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Signal sequence of human preproparathyroid hormone is inactive in yeast.

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Born W, Freeman M, Bornstein W, Rapoport A, Klein RD, Hendy GN, Khorana HG, Rich A, Potts JT Jr, Kronenberg HM.

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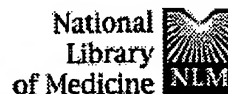
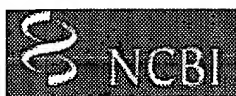
The biosynthesis of human preproparathyroid hormone (hpreproPTH) and the processing to mature parathyroid hormone (hPTH) was investigated in yeast. Cells were transformed with a plasmid that carried a fusion gene made of the yeast pyruvate kinase promoter, complementary DNA (cDNA) encoding a slightly modified form of hpreproPTH and the transcription termination signal from yeast triosephosphate-isomerase. In transformed yeast cells we identified a protein that was recognized by a PTH antiserum and, on gel electrophoresis, comigrated with hpreproPTH marker. The amino-terminal sequence of the protein was consistent with that of hpreproPTH, indicating that the hormone precursor is not processed. It was localized inside the cell, when analyzed in pulse-chase experiments by trypsin accessibility in intact and lysed spheroplasts. In contrast, when mRNA from these yeast cells and from human parathyroid tissue was translated into preproPTH in a reticulocyte lysate supplemented with canine pancreatic microsomes, the preproPTHs from both mRNAs were transported and cleaved with identical efficiencies. We conclude that hpreproPTH is synthesized in yeast but not recognized and processed like a precursor of a secreted protein by the yeast secretory apparatus.

PMID: 3455619 [PubMed - indexed for MEDLINE]

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☐ 1: Mol Endocrinol 1987 Jan;1(1):5-14[Related Articles, Links](#)

Human preproparathyroid hormone synthesized in Escherichia coli is transported to the surface of the bacterial inner membrane but not processed to the mature hormone.

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Born W, Freeman M, Hendy GN, Rapoport A, Rich A, Potts JT Jr, Kronenberg HM.

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cDNA encoding human preproPTH (hpreproPTH) was expressed in Escherichia coli to study the processing of the precursor to hPTH and its secretion by the bacterial secretory apparatus. We first constructed hybrid genes that differed randomly in the distance between the E. coli lac promoter's ribosomal binding site and DNA encoding a fusion protein with beta-galactosidase activity and the prepro sequence of hpreproPTH on the aminotermminus. Starting with clones identified as efficient producers of beta-galactosidase on indicator agar plates, the coding sequence for hpreproPTH was reconstituted intact. In a different construction we placed the hpreproPTH coding sequence downstream from the lac promoter at a distance of 12 base pairs from the ribosomal binding site. PTH immunoreactive proteins from multiple clones were identified by protein gel electrophoresis and by protein microsequencing. PTH-related proteins encoded by different plasmids were shown to be hpreproPTH with amino-terminal extensions of either two or four amino acids and as authentic hpreproPTH. Two hPTH fragments, hPTH(3-84) and hPTH(8-84), were also observed. The trypsin accessibility of hpreproPTH and of the two hPTH fragments in pulse-chase, cell-fractionation experiments using intact and lysed spheroplasts lets us conclude that the mammalian signal sequence directs hpreproPTH to the surface of the spheroplast membrane but is not appropriately cleaved by the signal peptidase.

PMID: 3331711 [PubMed - indexed for MEDLINE]